Journal of Visualized Experiments

Using Dynamic, Interactive Paradigms to Study Social Neuroscience in Rhesus Macaque Monkeys

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58755R3
Full Title:	Using Dynamic, Interactive Paradigms to Study Social Neuroscience in Rhesus Macaque Monkeys
Keywords:	Nonhuman primates; social interaction; Neuroscience; neuroeconomics; dominance; competition
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Open Access (US$4,200)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Philadelphia, Pennsylvania, United States

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TITLE:

Using Dynamic, Interactive Paradigms to Study Social Neuroscience in Rhesus Macaque Monkeys

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KEYWORDS:

nonhuman primates, social interaction, neuroscience, neuroeconomics, dominance, competition

SUMMARY:

Here we present two protocols to examine the neuronal mechanisms underlying live interactions between pairs of rhesus macaque monkeys, in an effort to gain insight into the neurobiology of complex social behaviors in humans.

ABSTRACT:

Humans and nonhuman primates exhibit complex and dynamic behaviors during social interactions. Laboratory studies, however, typically probe social cognition using simplified, discretized tasks, and rarely examine the interactions between two live agents in real-time. To address this gap, we describe two experimental paradigms featuring pairs of rhesus macaque monkeys interacting in multidimensional decision spaces. First, we describe a task-free face-off paradigm used to study the effects of neuropeptides on dominance, an essential building block of macaque society that color all other aspects of social interactions. Then, a virtual soccer game is used to examine the neuronal mechanism of strategic competition, an important part of animals' fight for survival when faced with limited resources. Utilizing these innovative paradigms, in combination with an array of behavioral and physiological measurements, we have uncovered how different key regions within the social brain network contribute to the complex behaviors observed in natural social interactions, and how neuropeptides modulate such behaviors.

INTRODUCTION:

Many animals, including humans, have evolved to live among large groups of conspecifics. Thus, various aspects of our everyday experience and consciousness are social in a most fundamental sense, as they are often determined by and directed against inputs from other people[1-2]. This social experience can be perceptual, such as recognizing a face or comprehending a speech, or more abstract and strategic, such as deciding whether to attend a party and, if at the party, whether to talk to someone or to avoid them. Social neuroscience examines the neuronal underpinning of these perceptual and decision-making processes. Many early studies in social neuroscience adopted a representationalism perspective[3-4] and treated social cognition as an internalized process within an individual. As such, these studies predominantly featured individual subjects responding to "social" stimuli such as pictures or videos of other conspecifics. Even though such paradigms successfully identified a number of key brain areas contributing to social cognition[5], it is unclear how these areas mediate everyday social interactions such as trusting, betraying, questioning, deceiving, competing, cooperating, bargaining, and offering, all of which require attending to the inputs from other people in real-time.

Some studies have aimed at filling this gap by using interactive behavioral paradigms inspired by or borrowed from game theory[6], such as choosing between betraying or cooperating with someone[7-8] or deciding whether to donate or invest in someone[9-14]. While preserving certain features of interpersonal interactions, these studies still feature discrete, static, often binary choices that do not necessarily reflect the complexity of real-life interactions[15-16], as the latter often occur within an evolving decision space that is continuously reshaped by an individual's own behavior and the behavior of other individuals.

To overcome these limitations, here we report two studies in which pairs of rhesus macaque monkeys interact in multidimensional decision spaces. Much like humans, macaques live in large, hierarchical, mixed-sex groups[17] and use visual and vocal signals to communicate social information to each other[18-20]. These behaviors are mediated by a network of cortical and subcortical brain areas homologous with the human social brain network[21-23]. Together, these factors make macaque monkeys the ideal animal model for studying the neurobiology of social cognition and social deficits associated with psychiatric disorders[24-26]. In the first study, a task-free "face-off" paradigm is used to probe how neuropeptides oxytocin (OT) and arginine vasopressin (AVP) affect naturalistic, spontaneous behaviors in monkeys[27]. In the second study, the behavior and attendant physiology of monkeys playing a two-player competitive "soccer" game (aka "penalty kick") is examined. We found that different key regions within the social brain network, namely the anterior cingulate gyrus (ACCg) and the mid-superior temporal sulcus (mSTS), significantly contribute to the naturally complex behaviors observed in social interactions, and neuropeptides OT and AVP can modulate such behaviors.

PROTOCOL:

All procedures reported in this study were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and performed in accordance with their relevant guidelines and regulations.

1. Experiment 1: Modulation of Spontaneous Social Interaction and Flattening of Dominance Hierarchy with Oxytocin and Vasopressin

NOTE: The entirety of this experimental protocol has been described previously in detail[27].

1.1. Animals

1.1.1. Use adult rhesus macaque monkeys (*Macaca mulatta*) for this experiment.

NOTE: For the experiment described here, the participants are seven monkeys from the same colony room.

1.1.2. Ensure that, for the duration of the experiment, all monkeys occupy the same colony room and are in continuous visual and auditory contact with each other within the colony.

1.1.3. Ensure that all animals have settled in the colony for at least half a year before starting the experiment; do not introduce new members into the colony for the duration of the experiment.

1.2. Experimental set-up

1.2.1. In each experiment session, place two monkeys in their respective primate chairs and position the two chairs close together (~30 cm apart from edge to edge) so that the monkeys can see each other face-on without touching.

1.2.2. Position a video camera (60 fps) on the right side of M1/the left side of M2 to simultaneously record both monkeys' behaviors for 5 min.

NOTE: M1 is defined as the monkey that receives the pharmacological treatment each day; M2s are all the other monkeys who do not receive treatment for that day.

1.2.3. On each day, ensure one M1 faces all different M2s (six in total) sequentially, each for 5 min. In addition, ensure that the same M1 faces an empty chair for 5 min as a nonsocial control. Ensure that the order in which M1 faces the M2s and the empty chair is determined randomly each day.

1.3. Pharmacological manipulation

1.3.1. Inhalation

NOTE: The detailed procedure for the intranasal OT delivery in macaque monkeys has been described previously[14].

1.3.1.1. Briefly, first train monkeys to accept a pediatric nebulizer mask over the nose and mouth through positive reinforcement.

1.3.1.2. On each day, deliver 1 mL of OT (25 IU/mL in saline), AVP (25 IU in 1 mL of saline), or saline through the nebulizer at a constant rate (0.2 mL/min) over a total of 5 min (only to M1, not M2s).

1.3.1.3. Ensure that the neuropeptide and saline treatments are delivered on alternating days, with each monkey receiving no more than five treatments per week.

1.3.1.4. Make sure that the video recording is done 30 - 120 min after the intranasal delivery.

1.3.1.5. Repeat each treatment 5x in each M1.

1.3.2. Injection

NOTE: The procedure for the OT injection has also been described previously[28].

1.3.2.1. Briefly, first identify the location of ACCg through structural magnetic resonance images (MRI; 3Tesla (T), 1 mm slices) and then confirm it with electrophysiological recordings.

1.3.2.2. On each day, restrain the subject monkey *via* a head holder fixed on the primate chair, and inject 2 µL of OT (0.5 IU/µL of OT in saline), AVP (1 IU in 2 µL of saline), or saline into the ACCg *via* a microsyringe (only to M1, not to M2s). To minimize tissue damage, make sure all injections are delivered at a rate of 0.2 µL/min and restricted unilaterally in each animal.

1.3.2.3. Ensure that the neuropeptide and saline injections are delivered on alternating days, with each monkey receiving no more than one injection every other day.

NOTE: Do not inject OT and AVP in the same monkey within the same week.

1.3.2.4. Repeat each treatment 5x in each M1.

2. Experiment 2: Examination of Strategic Competition with a Virtual Soccer Game

2.1. Animals

2.1.1. For the competitive soccer game, train at least two adult macaque monkeys as kicker and two as goalie (see below for the initial training).

2.1.2. Ensure that, for the duration of the experiment, all monkeys occupy the same colony room and are in continuous visual and auditory contact with each other within the colony.

2.2. Behavioral paradigm

2.2.1. General set-up

2.2.1.1. Position a monkey player in a primate chair with its head restrained and facing a 120 Hz LCD monitor (27 inch) 60 cm away, with access to a joystick mounted in a clear box attached to the front of the chair.

2.2.1.2. Ensure that the monkeys can look around throughout the experiment without turning their heads. Record the eye movements and pupil size changes with an infrared eye-tracking system (1,000 Hz) mounted on the primate chair.

2.2.1.3. Use a computer running software (*e.g.*, MATLAB) and Psychtoolbox to control all aspects of the experiment, including displaying visual stimuli on the monitor, sampling continuous input from the joystick (at 120 Hz), and opening and closing solenoid valves to dispense juice rewards.

2.2.1.4. Use Psychtoolbox to render three stimuli on the screen—the ball (controlled by the kicker), the bar (controlled by the goalie), and the goal line—against a black background. Luminance-balance all stimuli on the screen by adjusting the corresponding RGB values.

2.2.1.5. At the beginning of each trial, render the ball and the bar in the middle of the screen. Ensure that the ball is on the left and the bar on the right in front of the finish line. Throughout the trial, render the ball across the screen (left to right) at a constant horizontal speed (~350 pixels/s).

2.2.1.6. Train the kicker to use the joystick to move the ball vertically to bypass the goalie. Train the goalie to use the joystick to move the bar vertically to block the ball (see section 2.2.2). Ensure the Y-axis speeds of the ball and the bar are constant, and that the joysticks only control the direction of the movement.

2.2.1.7. At the end of each trial, deliver a juice reward to the winner (~0.8 mL) but not to the loser. Ensure the juice tube is positioned with the mouthpiece attached to the primate chair and within the monkey’s reach.

2.2.2. Initial behavioral training

2.2.2.1. First, train the monkeys on the virtual soccer game by making them play with a computer opponent exclusively. Ensure that the computer kicker and goalie follow very simple movement algorithms and always move in straight lines (with no redirections).

NOTE: This stage of training is classic behavior shaping.

2.2.2.2. When training the goalie, first make the ball go in one direction every time (*e.g.*, 45° up), and reward the monkey for any movement in the right direction, until it gradually approaches and finally catches the ball. Then, change the ball direction and repeat the process. When training the kicker, systematically make the goalie bar block off different portions of the screen and reward the monkey for any movement away from the goalie bar.

2.2.2.3. Train the monkeys with the same algorithms until their performance reaches at least an 80% win rate against a computer opponent in all movement directions.

2.2.3. Live-play condition

NOTE: All experiments should be carried out in a dimly lit room (20 - 35 cd/m^2) to ensure the visibility of the opponent.

2.2.3.1. Refer to section 2.2.1 for the general experimental set-up, including monitors, programs, monkey restraint, and eye tracking.

2.2.3.2. During the live-play condition, position the two players at opposite corners of the room, allowing both visual access to their opponent. Place a monitor displaying the visual stimuli in front of each player (60 cm). In other words, ensure that each player faces a monitor directly ahead and an opponent at a 45° angle.

2.2.3.3. Throughout the experiment, use a video camera mounted on top of each monitor to record the facial and hand movements of the monkey facing that monitor.

2.2.3.4. Adjust the length of the goalie bar slightly (ranging 250 - 350 pixels) to ensure an average win rate of 35% - 65% for both players. Ensure that the colors, speeds, dimensions, and starting positions of the ball bar and the goalie bar are consistent across all sessions.

2.3. Electrophysiological recording

2.3.1. Localization of the mid-superior temporal sulcus

2.3.1.1. Use structural magnetic resonance images (MRI, 1 mm slices) to guide the placement of recording tracks (see Jiang and Platt[27] and Chang *et al.*[28] for more detailed descriptions of MRI methods).

NOTE: A mask consisting of a 3 mm sphere around a seed at the fundus of the STS was made (X = 18.75, Y = -10.00, Z = -2.25, according to the Montreal Neurological Institute [MNI] atlas), and this choice was based on previous research indicating that this region of mSTS exhibits a functional connectivity profile most similar to the human temporoparietal junction (TPJ)[29].

2.3.1.2. Convert the mask to the individual monkey’s native-space structural scan to identify the target recording location.

2.3.2. Single-unit recording

NOTE: Single-unit recording should be performed while the monkeys are actively competing with each other in the virtual soccer game.

2.3.2.1. Make single-unit recordings with either single tungsten electrodes or multichannel linear arrays.

2.3.2.2. On each day, restrain the subject monkey *via* a head holder fixed on the primate chair. Secure a sterilized single electrode or electrode array onto the recording chamber *via* an X-Y stage and an adapter.

2.3.2.3. Penetrate the dura using a sterilized guide tube (22 G, stainless steel), and lower the electrode through the guide tube *via* a hydraulic microdrive.

2.3.2.4. Filter and record multiunit activity, as well as local field potentials (LFPs). Isolate the activity of single neurons offline using principal component analysis (PCA) and other sorting algorithms in three-dimensional feature space.

REPRESENTATIVE RESULTS:

Experiment 1. Modulation of Spontaneous Social Interaction and Flattening of Dominance Hierarchy with Oxytocin and Vasopressin:

Most prior studies have focused on examining how neuropeptides modulate performance in arbitrary laboratory tasks, thus limiting ecological validity and translational potential. As both OT and AVP are being extensively investigated for their therapeutic potentials in improving daily social interactions and communications, we decided to test their effects on spontaneous, naturally occurring social behaviors. We recorded a series of 5 min-long videos of pairs of adult male macaque monkeys interacting with each other in close proximity (see **Figure 1A**, as well as **Animated Figure 1**). Prior to each session, one monkey (hereafter M1) inhaled either saline or OT *via* a pediatric nebulizer, whereas the other monkey (hereafter M2) did not receive any treatment. Both monkeys' behaviors were rated by independent observers and subsequently converted to a set of ethograms (see **Figure 1B,C** for example ethograms and a summary of quantifiable behaviors from one monkey pair in four consecutive saline sessions).

The results of this study have been published in their entirety[27]. Briefly, the overall effect of OT across the population was a small reduction in staring by the treated monkey (**Figure 2A**), as well as in his untreated partners (**Figure 2B**). As a direct stare is considered dominant behavior among male monkeys, a decrease in mutual staring indicates a relaxation of social interaction under OT treatment. Note that the monkeys' staring behavior at an empty chair remained unchanged, indicating that the effect of OT was specifically social (**Figure 2B**, inset). Across all possible monkey pairs (n = 7 x 6 = 42), the difference between the staring durations of M1 and M2 varied greatly, reflecting the existing social hierarchy (**Figure 2C**). OT narrowed this gap and diminished the difference in staring durations, suggesting a flatter dominance hierarchy (**Figure 2D**).

Due to a strong similarity in molecular structure, OT can bind to AVP receptors with high affinity and *vice versa*[30-32]. Thus, delivering either OT and AVP at a high concentration may evoke behavioral changes by recruiting both neuropeptide systems. To address this, the effects of OT and AVP inhalation were systematically compared at the same concentration in the same subjects (25 IU delivered in a 1 mL saline vehicle). We found that, just as OT, AVP inhalation significantly reduced the staring by the treated monkeys, as well as by their untreated partners. Overall, AVP was more effective than OT in reducing staring by all monkeys. Notably, in neither condition did the treatment alter the monkeys' staring behavior at an empty chair. Across all monkey pairs in this dataset ($n = 3 \times 6 = 18$), OT and AVP similarly flattened the pre-existing social hierarchy (**Figure 2E**).

Finally, we investigated the neural circuitry mediating the behavioral effects of OT and AVP. To do so, OT or AVP (1 IU in a 2 µL saline vehicle) was injected focally into the ACCg, a subregion of ACC strongly implicated in social behavior[33]. We found that just as with the inhalation treatments, both OT and AVP injections significantly reduced the staring by the treated monkeys, as well as by their untreated partners, without altering their staring behavior at an empty chair. Across all monkey pairs ($n = 2 \times 6 = 12$), both OT and AVP injections also effectively flattened the existing social hierarchy (**Figure 2F**).

Experiment 2. Examination of Strategic Competition with a Virtual Soccer Game:

In this experiment, the behaviors of monkey kickers playing virtual soccer against conspecific goalies were studied. Kickers used a joystick to move a ball across the screen (left to right) to reach the goal line; goalies used a joystick to move a goalie bar to try to block the ball. A trial ended when either the kicker maneuvered the ball to the goal line or the goalie intercepted the ball (see **Animated Figure 2** for recorded live trials). **Figure 3A** depicts representative trajectories of kicker and goalie movements in a 25-trial block, during which both players won with comparable probabilities. Although each trial began exactly the same way and ended with a simple, symmetric, zero-sum payoff, the task elicited complex, dynamic interactions between pairs of monkeys.

This complexity in competitive behavior was reflected in the variability of response times (RT, defined as the time of the first move made by the player) for both kickers and goalies across trials (**Figure 3B**). On most trials, however, goalies lagged kickers by a short delay (kicker = 0.60 ± 0.01 s, goalie = 1.00 ± 0.01 s, $r = 0.17$, $P = 0.000$). In addition, kickers often changed their trajectories online (hereafter 'redirections') to increase their chances of bypassing the goalie. A higher redirection rate was correlated with winning for the kicker but not for the goalie (the kicker redirection rate on losing trials = 0.79 ± 0.01, winning trials = 0.93 ± 0.01; the goalie redirection rate on losing trials = 0.80 ± 0.01, winning trials = 0.68 ± 0.01) (**Figure 3C**). Overall, goalies tended to redirect in response to kicker redirections, consistent with a reactive strategy ($r = 0.46$, $P = 0.000$). Furthermore, both kickers and goalies varied in their movement directions across trials (**Figure 3D**). Just like penalty kicks in real soccer[34-38], goalies tended to do better when their movements perfectly mirrored those of the kicker (diagonal line, upper left to lower right corner, kicker win rate = 0.31). Nevertheless, not all movement combinations were equally effective. Generally, monkeys were able to align their plays with the probabilities of winning. For example, monkey kickers were less likely to win when their final shot was downwards (second and fourth columns, kicker win rate = 0.37) and, in fact, kickers were less likely to shoot downwards (48.2%) than upwards (51.8%). Additionally, as in real soccer, the kicker performance was positively correlated with the variability of ball end point ($r = 0.32$, $P = 0.000$).

In summary, the relatively unconstrained movements from pairs of players in the task presented here can be reduced to a set of quantifiable behavioral parameters that define each player's strategies. Although the final outcome of the game is a result of the interaction between two players, the kicker and the goalie clearly benefit from very different strategies. In general, kickers win by being unpredictable, while goalies win by quickly and precisely responding to the kickers' actions. It is worth noting that, as the same two players competed against each other over and over again, they relied more heavily on one strategy or the other at different time points. Most importantly, changes in the goalie's strategy always closely followed changes in the kicker's strategy (**Figure 3E-G**), illustrating the mutually evolving nature of this behavioral paradigm.

During gameplay, we also recorded neuronal activity in mSTS, an area of the macaque brain long associated with social perception[39-43] and recently identified as the potential homolog of human TPJ[29]. We found that, in both kickers and goalies, mSTS neurons signaled the complexity of play, as well as trial outcomes (*n* = 133 mSTS neurons in kicker, 125 mSTS neurons in goalie) (**Figure 4A**). During the task performance, the kicker's mSTS population showed increased activity, whereas the goalie's mSTS population showed suppression (kicker: 41/133 = 30.8% neurons were significantly modulated, among which 80.5% were excitatory; goalie: 28/125 = 22.4% neurons were significantly modulated, among which 67.9% were suppressive). During and, especially, after reward delivery, in both kickers and goalies, the mSTS activity was significantly elevated above baseline (kicker: 55/133 = 41.4% neurons were significantly modulated, among which 72.7% were excitatory; goalie: 97/125 = 77.6% neurons were significantly modulated, among which 96.9% were excitatory). We also found that, during task performance, the neuronal activity in the kicker's mSTS significantly distinguished whether any redirections were made (firing rate with no redirections = 18.42 ± 0.25 spikes/s, with redirections = 22.03 ± 0.29 spikes/s) (**Figure 4B**, upper panel). By contrast, the neuronal activity in the goalie's mSTS was not correlated with the number of redirections made but, instead, was correlated with the response time of the goalie relative to the kicker (the firing rate when the goalie moved before the kicker = 31.77 ± 0.28 spk/s, when the goalie moved after the kicker = 28.54 ± 0.42 spk/s) (**Figure 4B**, lower panel). During the reward epoch, by contrast, both the kicker's and the goalie's mSTS activities were elevated for winning over losing (kicker: 54.1% neurons distinguished winning from losing, among which 93.1% preferred winning; goalie: 84.0% neurons distinguished winning from losing, among which 89.5% preferred winning) (**Figure 4C, D**).

Together, these findings demonstrate that mSTS activity correlates with various aspects of dynamic, strategic social interactions. During task performance, mSTS activity covaries with the level of effort exerted by the kicker, as well as the responsiveness of the goalie to the kicker. During and after reward delivery, mSTS neurons most reliably signal winning over losing. To our knowledge, these are the first abstract, strategic, nonperceptual social signals reported in the primate mSTS.

FIGURE AND TABLE LEGENDS:

Figure 1: Ethograms and quantifiable behaviors. (**A**) This panel shows the experimental design.

The top shows the treatment administration: one monkey (M1) received saline, OT, or arginine-vasopressin (AVP) treatments *via* intranasal nebulization or intracortical injection. The bottom shows the experimental set-up (left: top-down view; right: side view). After receiving the treatment, the same M1 faces another monkey (M2) or an empty chair for 5 min in close proximity, with a camera on the side recording all behaviors to a video file. (**B**) This panel shows an example set of ethograms from one monkey pair facing each other in four saline sessions. M1 (Sh) inhaled saline; M2 (E) did not. (**C**) This panel shows a summary of behaviors demonstrated by the same M1 (Sh) facing different M2s under the influence of saline. X-axis: M2 identities, ordered by the time M1 spent staring at each. Y-axis (left): average M1 behavior (*i.e.*, staring, looking away, turning away) durations. Y-axis (right): average M1 behavior (*i.e.*, yawning) frequency. This figure has been modified from Fig. 1 of Jiang and Platt[27].

Figure 2: OT and AVP inhalation and injection modulate the pre-existing social hierarchy. (**A**) Overall, OT treatment reduces staring by M1 (*n* = 7 male monkeys, 180 face-off sessions x 2 treatment conditions). The black line illustrates the gamma fit of the saline distribution, the magenta line the gamma fit of the OT distribution. The arrows indicate medians. (**B**) OT treatment also reduces the overall staring time by M2. The black line illustrates the gamma fit of the saline distribution, the magenta line the gamma fit of the OT distribution. The arrows indicate medians. The **insert** shows that, compared with saline (grey), OT (pink) does not change M1's staring at an empty chair. The error bars = the mean ± the standard error of the mean (SEM). (**C**) As M1's staring increases, M2's staring decreases, and the difference between the two widens correspondingly. X-axis: monkey pairs ordered according to the difference between M1's and M2's staring times; left to right corresponds to an increasing dominance of M1 over M2. (**D**) OT inhalation narrows the gap between the staring durations of M1 and M2 and flattens the dominance hierarchy. X-axis: monkey pairs in the same order as in panel **C**. (**E**) In a different monkey colony (*n* = 3 male monkeys as M1s, 3 male and 4 female monkeys as M2s, 90 face-off sessions x 3 treatment conditions), both OT and AVP inhalations reduce the difference between M1's and M2's staring. X-axis: monkey pairs ordered by the difference in staring between M1 and M2 under saline; left to right corresponds to an increasing dominance of M1 over M2. (**F**) Both OT and AVP injections in the ACCg decrease the difference between M1's and M2's staring (*n* = 2 male monkeys as M1s, 3 male and 4 female monkeys as M2s, 60 face-off sessions x 3 treatment conditions). X-axis: monkey pairs ordered by the staring difference between M1 and M2 under saline; left to right corresponds to an increasing dominance of M1 over M2. This figure has been modified from Fig. 2, 4, and 5 of Jiang and Platt[27].

Figure 3: Monkeys display complex strategies in the virtual soccer game. (**A**) This panel shows the movement trajectories of the kicker and the goalie in 25 consecutive trials. The ball (blue) moves across the screen from left to right with a fixed horizontal speed; the kicker only controls the ball's movement vertically. The goalie only controls the goalie bar (red) vertically. X-axis: time. Y-axis: The Y-axis on the screen. For this display, the goalie's trajectory is compressed along the X-axis. Crosses mark the response time (defined as the time of the first movement of the ball or bar); circles mark any redirections (defined as sudden (<0.1 s) changes in the ball or bar direction). The blue lines illustrate kicker trajectories in losing trials, the cyan lines kicker trajectories in winning trials, the red lines goalie trajectories in losing trials, and the pink lines goalie trajectories

in winning trials. The white line illustrates the finish line. (**B**) This scatterplot shows the response times of the kickers and goalies in all live trials. (**C**)This panel shows the average number of redirections made per trial in winning and losing trials. The error bars = the mean ± the SEM. (**D**) This table shows the number of trials (black) and the associated win rate (kicker, blue) in each movement direction combination. Kicker left-to-right columns and goalie top-to-bottom rows: started upward and continued, started upward and finished down, started upward and ended down, started downward and continued. Blue cells show the kicker win rate >0.5 and pink cells the goalie win rate >0.5. (**E**) This graph shows the correlation between response time-related strategies and monkey performance (measured as the correlation between the kicker response time and the kicker win rate, and the goalie-kicker response time and the goalie win rate in every 100 trials). X-axis from left to right indicates the time. (**F**) This graph shows the correlation between redirection-related strategies and monkey performance (measured as the correlation between the kicker redirection rate and the kicker win rate, and the goalie-kicker redirection rate and the goalie win rate in every 100 trials). X-axis from left to right indicates the time. (**G**) This graph shows the kicker correlations from panels **E** and **F** plotted together to show the switches between strategies.

Figure 4: mSTS neurons signal strategic information and trial outcomes during live competition. (**A**) This graph shows the population peristimulus time histograms (PSTHs) for all mSTS neurons recorded during live trials, segregated by the trial outcome and aligned with trial onset (normalized to the pretrial baseline). The thin grey lines indicate the beginning and end of the gameplay. Reward delivery starts at the end of the gameplay and lasts 0.8 - 1 s, as indicated by the thick grey line. The thickness of each line indicates the mean ± the SEM. (**B**) The top panel shows the firing rate distribution (z-scored to the pretrial baseline) for the kicker's neurons during the task epoch, segregated by whether any redirections were made by the kicker. The bottom panel shows the firing rate distribution (z-scored to the pretrial baseline) for the goalie's neurons during the task epoch, segregated by whether the goalie moved before or after the kicker. (**C**) This panel shows the firing rate distribution (z-scored to the pretrial baseline) for all kicker neurons during the reward epoch, segregated by the trial outcome. (**D**) This panel shows the firing rate distribution (z-scored to the pretrial baseline) for all goalie neurons during the reward epoch, segregated by the trial outcome.

Animated Figure 1: Face-off. A brief video clip (~1 min) taken from one experimental session. M1 (D, left) inhaled OT prior to the experiment, whereas M2 (S, right) did not receive any treatment. A variety of behaviors can be identified from the video clip, including (not necessarily in this order) staring, lip-smacking, looking away, turning away, and yawning.

Animated Figure 2: Penalty kick. Ten consecutively recorded trials of a monkey kicker playing against a monkey goalie. X-axis: The X-axis on and off screen (normalized). Y-axis: The Y-axis on screen (normalized). The white line is the finish line, the green circle the kicker eye position, and the magenta circle the goalie eye position. The green rectangle indicates the physical location of the kicker and the magenta rectangle indicates the physical location of the goalie.

DISCUSSION:

This study reported on two interactive paradigms, both featuring pairs of nonhuman primates responding to each other's behaviors and modifying each other's behavioral environment in real-time. The 'face-off' paradigm was used to examine the effects of neuropeptide treatment on social dominance, whereas the 'penalty kick' paradigm was leveraged to investigate how primate mSTS contributes to strategic competition. While the neurobiological questions motivating these studies are very different, both behavioral paradigms aim to improve upon the existing experimental designs in social neuroscience by providing the subjects with a more dynamic and evolving decision space. In the following sections, the potential applications, theoretical impact, and limitations to these two paradigms will be discussed.

Experiment 1. Modulation of Spontaneous Social Interaction and Flattening of Dominance Hierarchy with Oxytocin and Vasopressin:

The current study shows that treating a group of male macaque monkeys intranasally with aerosolized OT relaxes their spontaneous social interactions with others and flattens the preexisting status hierarchy among monkeys. These results lend further support to the positive link between OT and social behavior observed in a variety of animals, from rodents[44-49] to nonhuman primates[14,28,50-55]. In contrast, the functional involvement of the OT system in human behavior, especially the effect of acute OT administration, remains highly controversial[56-60]. This substantial disparity between animal and human OT research may be deeply rooted in the fact that OT often has task- or stimulus-specific effects in humans[61-65], and it can interact with individual traits such as gender, personality, attachment styles, and psychopathology[66-69], making it difficult to compare across studies[70-71]. In this study, we chose to exclusively examine naturalistic social interactions among adult male macaque monkeys belonging to a small, stable, hierarchical group. This unique combination of a task-free paradigm and a closed social group may have contributed to the detectability of OT effects.

Additionally, using the same paradigm, we also found that the intranasal delivery of aerosolized AVP reproduces the effects of OT with greater efficacy and that all behavioral effects are replicated when OT or AVP is injected focally into the ACCg. As most cortical areas in primates, including the ACCg, lacks OT receptors but is rich in AVP receptors[72], these results indicate that exogenous OT may shape social behavior partially through nonspecific binding with AVP receptors. These findings bear important implications for the future use of OT in basic as well as translational research, and further highlight the importance of using flexible, naturalistic behavioral paradigms to investigate the neurobiology of nonapeptides.

It is worth noting that the otherwise unconstrained paradigm presented here does require placing monkey pairs in respective primate chairs, thus limiting their ability to avoid or retreat from each other. However, because the monkeys' heads are unconstrained, they can still look away or turn away from each other. While this set-up may have, to some extent, inflated the naturally existing tension among monkeys, it is necessary to induce quantifiable confrontational and avoidant behaviors, especially given the time constraint that is faced when conducting behavioral testing post neuropeptide inhalation in multiple monkey pairs.

Experiment 2. Examination of Strategic Competition with a Virtual Soccer Game:

Like perceptual decision making[73-74] and neuroeconomics[75-78], most prior research on social decision making has employed binary choice tasks (*i.e.*, two-alternative forced choice [2AFC] tasks) (for examples, see Rilling *et al.*[79], Sanfey *et al*.[80], Lee[81], and Haroush and Williams[82]). By contrast, we presented a continuous choice space, necessitating behavioral modifications in real-time in response to the behavior of other agents. The strategies monkey players displayed in this task parallel the multidimensional dynamics of real-life competitions among humans, such as chess, poker, and soccer[34-37,83,84]. Furthermore, naturally motivated by competition, all monkeys learned this task very quickly (the average number of training trials administrated before recording: kicker = 1,550 trials in 8.5 days, goalie = 3,800 trials in 12.5 days), and the strategies of pairs of players evolved together throughout time, supporting the idea of an evolving decision space where the action of one player exerts immediate impact on the other and *vice versa*.

It remains controversial whether macaques or other nonhuman primates possess a full, multilevel theory of mind (ToM). It is unclear, for example, whether monkeys are able to understand and exploit the false beliefs of others[85-86]. In addition, studies of ToM in nonhuman primates often have trouble distinguishing behaviors arising from true understanding and reasoning from those simply acquired through reinforcement learning and deployed according to simple rules. The ease with which the penalty kick paradigm evokes complex, flexible, strategic behaviors affords researchers the rare opportunity of side-stepping the controversy surrounding ToM and directly investigating the neurobiology of strategic decision making without overtraining monkeys.

Neuroimaging studies have indicated that the human TPJ uniquely contributes to ToM or to mentalizing in a social context[87-92]. Prior neurophysiological recordings in STS have linked it to social perception[39-43] but, until now, there was no established experimental paradigm to examine neurophysiological signals associated with ToM in nonhuman primates. Using this behavioral paradigm, we found that in mSTS, a subregion of monkey STS that shares the most similar functional connectivity with the human TPJ[29], neurons multiplex various abstract, nonperceptual signals associated with strategic social interactions. These findings lend support to the hypothesis that mSTS is the simian homolog of the human TPJ and endorse the idea that multilevel intentional reasoning in humans has its origins in abstract computations that contribute to strategic social interactions in nonhuman primates[93-94].

ACKNOWLEDGMENTS:

The authors thank their lab managers, Monica Carlson at Duke University and Heidi Steffen at the University of Pennsylvania, as well as the Department of Laboratory Animal Resources at Duke University and the University Laboratory Animal Resources at University of Pennsylvania, for providing excellent animal care. The authors thank Jean-Francois Gariépy for envisioning and designing parts of the experiment, and Wei Song Ong and Geoff Adams for their advice on data analysis. This work was supported by R01MH095894, R01MH108627, R37MH109728, and a grant from the Simons Foundation (SFARI 304935, to M.L.P.).

DISCLOSURES:

The authors have nothing to disclose.

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Figure1

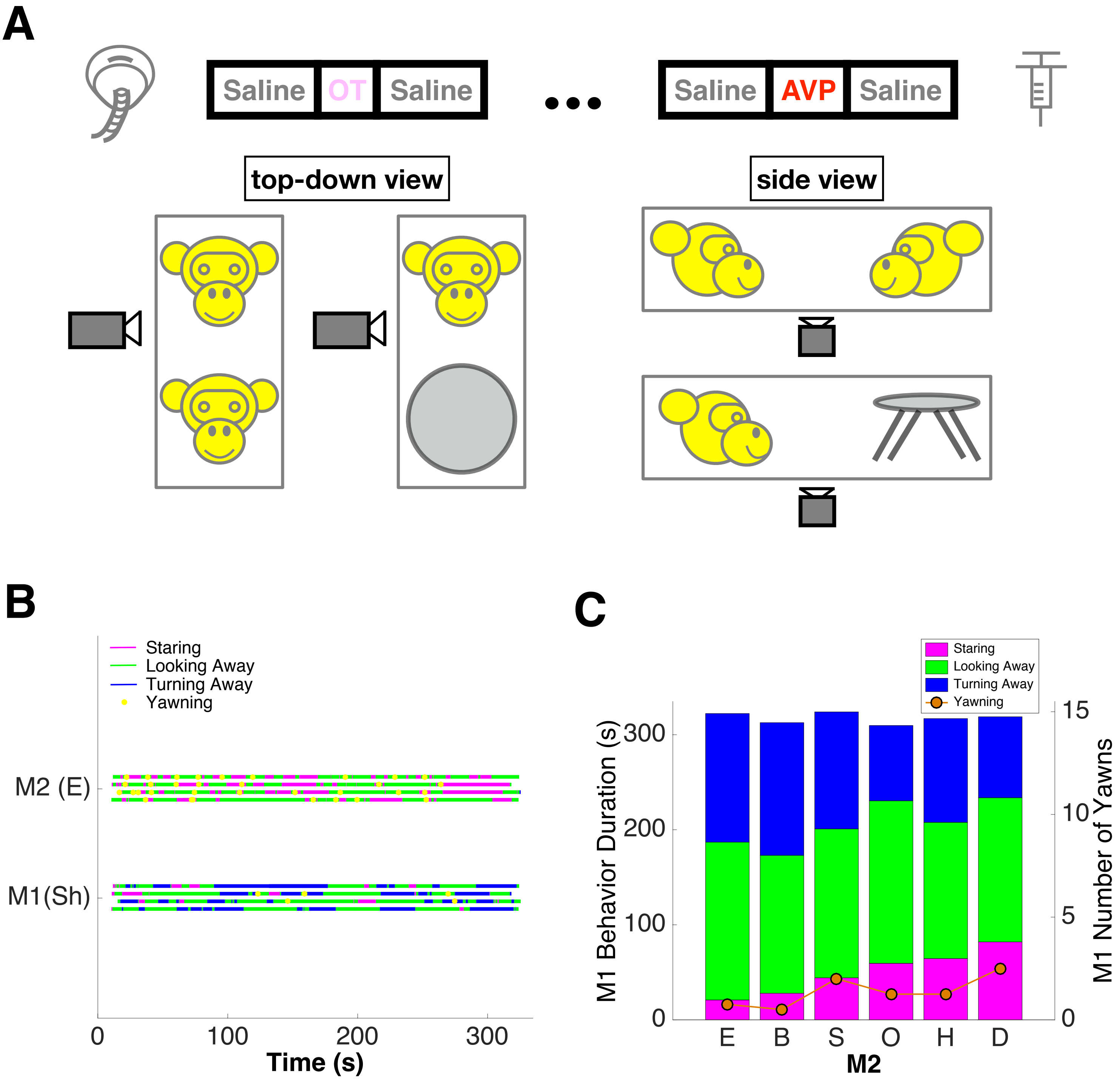
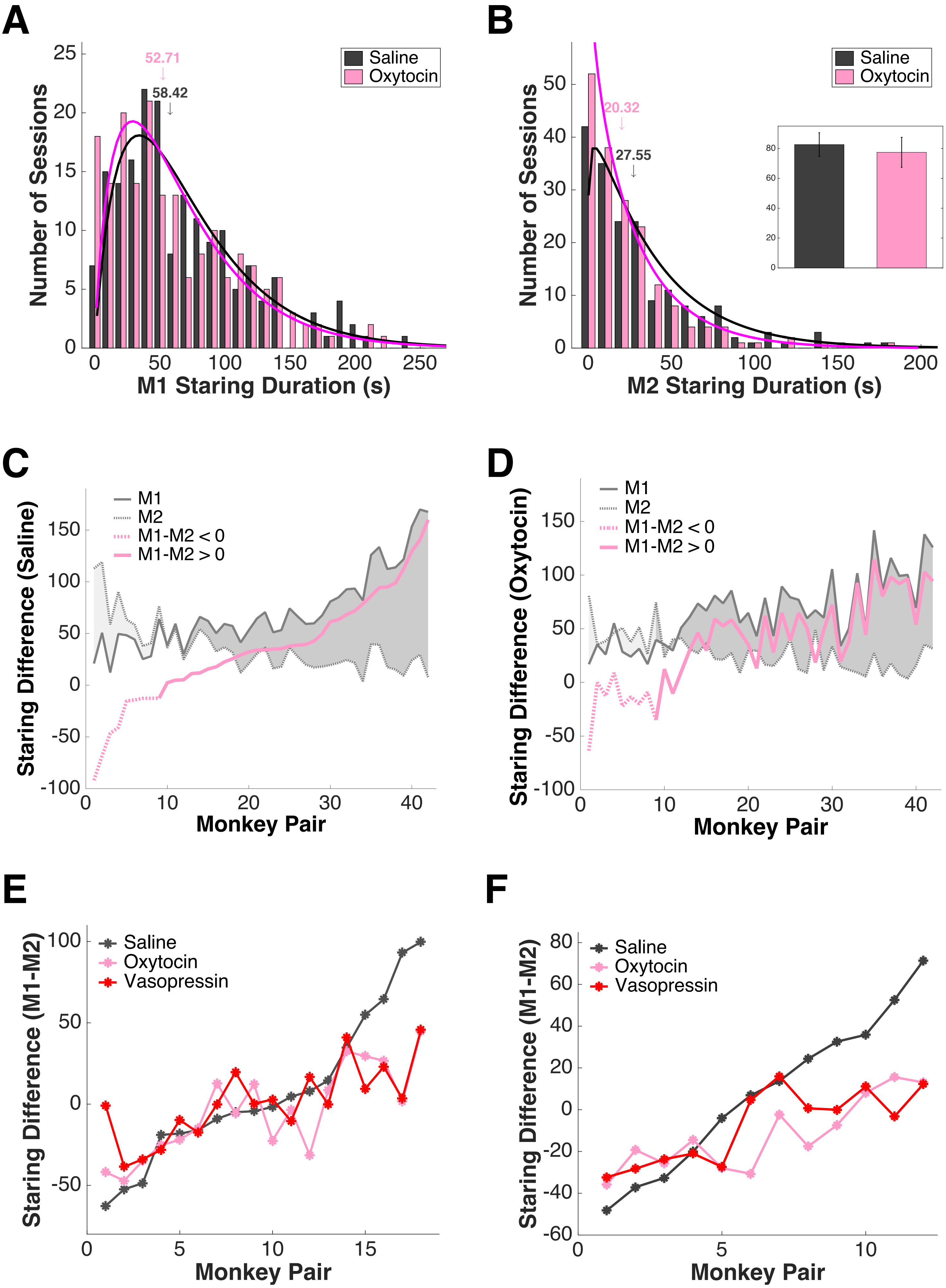
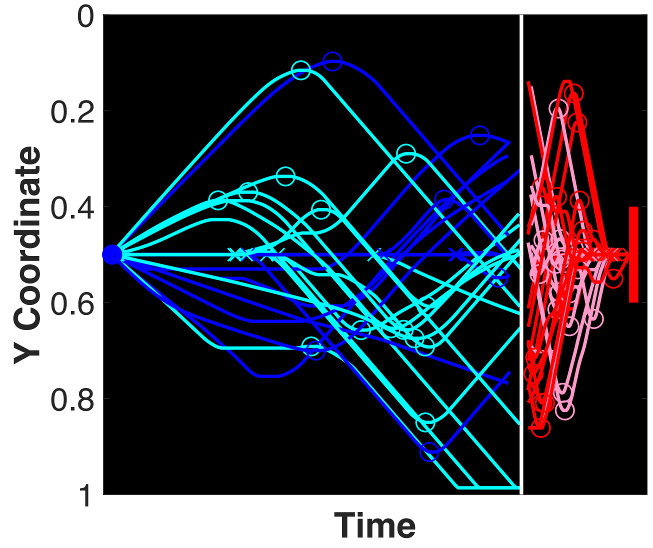

A
Saline
OT
Saline
Saline
AVP
Saline
top-down view
side view
B
Staring
Looking Away
Turning Away
Yawning
M2 (E)
M1(Sh)
0
100
200
300
Time (s)
C
Staring
Looking Away
Turning Away
Yawning
M1 Behavior Duration (s)
0
100
200
300
M1 Number of Yawns
0
5
10
15
E
B
S
O
H
D
M2

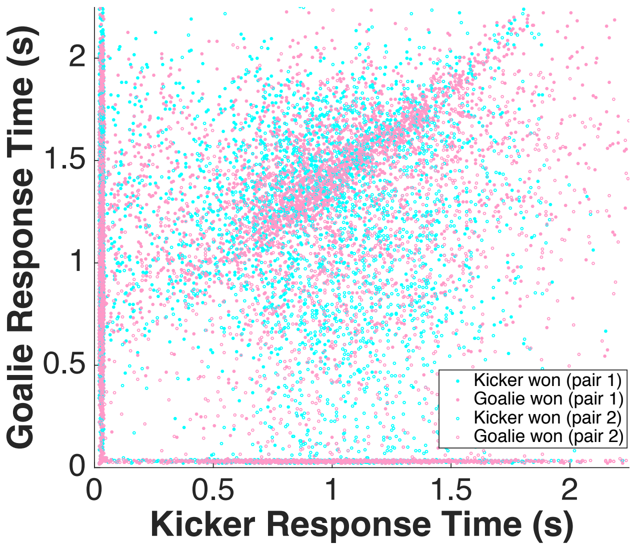
Figure2


A
Number of Sessions
M1 Staring Duration (s)
Saline
Oxytocin
52.71
58.42
0
5
10
15
20
25
0
50
100
150
200
250
B
Number of Sessions
M2 Staring Duration (s)
Saline
Oxytocin
20.32
27.55
0
10
20
30
40
50
0
50
100
150
200
0
20
40
60
80
C
Staring Difference (Saline)
Monkey Pair
M1
M2
M1-M2 < 0
M1-M2 > 0
-100
-50
0
50
100
150
0
10
20
30
40
D
Staring Difference (Oxytocin)
Monkey Pair
M1
M2
M1-M2 < 0
M1-M2 > 0
-100
-50
0
50
100
150
0
10
20
30
40
E
Staring Difference (M1-M2)
Monkey Pair
Saline
Oxytocin
Vasopressin
-50
0
50
100
0
5
10
15
F
Staring Difference (M1-M2)
Monkey Pair
Saline
Oxytocin
Vasopressin
-60
-40
-20
0
20
40
60
80
0
5
10

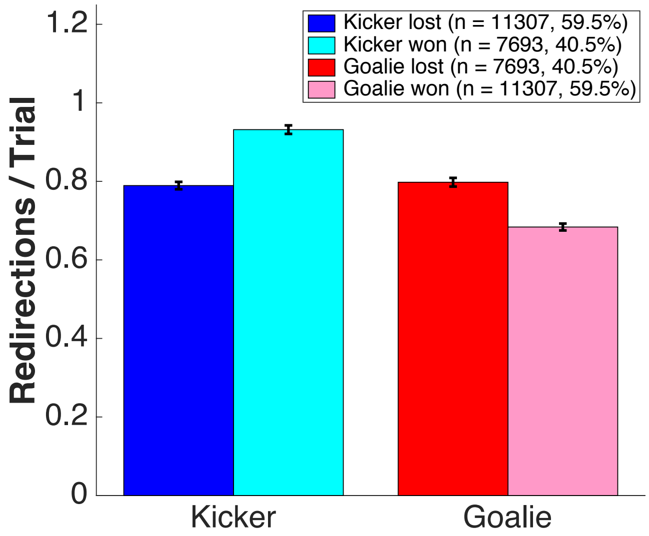
A



B



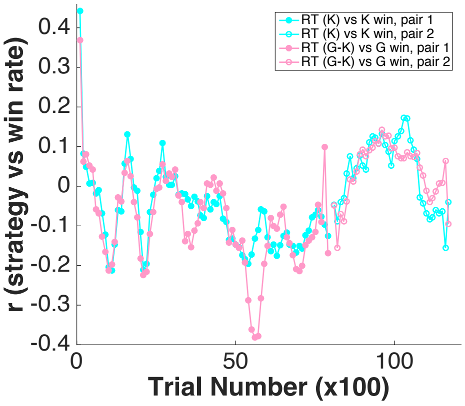
C



D

K \ G	up up	up down	down up	down down	all
up up	1874 0.34	294 0.63	525 0.24	174 0.64	2867 0.37
up down	170 0.73	338 0.29	159 0.41	730 0.44	1397 0.44
down up	906 0.55	83 0.61	1183 0.32	287 0.70	2459 0.46
down down	217 0.71	244 0.30	970 0.53	3446 0.30	4877 0.36
all	3167 0.45	959 0.42	2837 0.38	4637 0.36	11600 0.39

E



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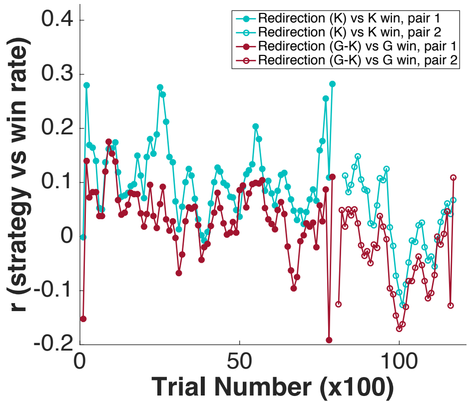

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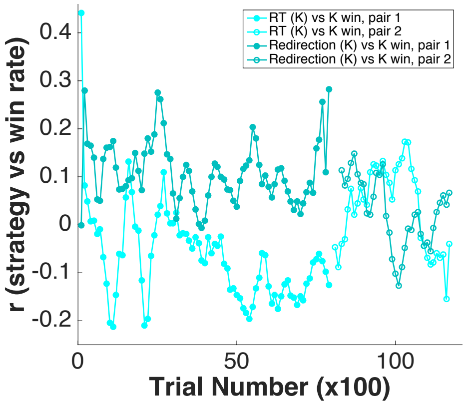
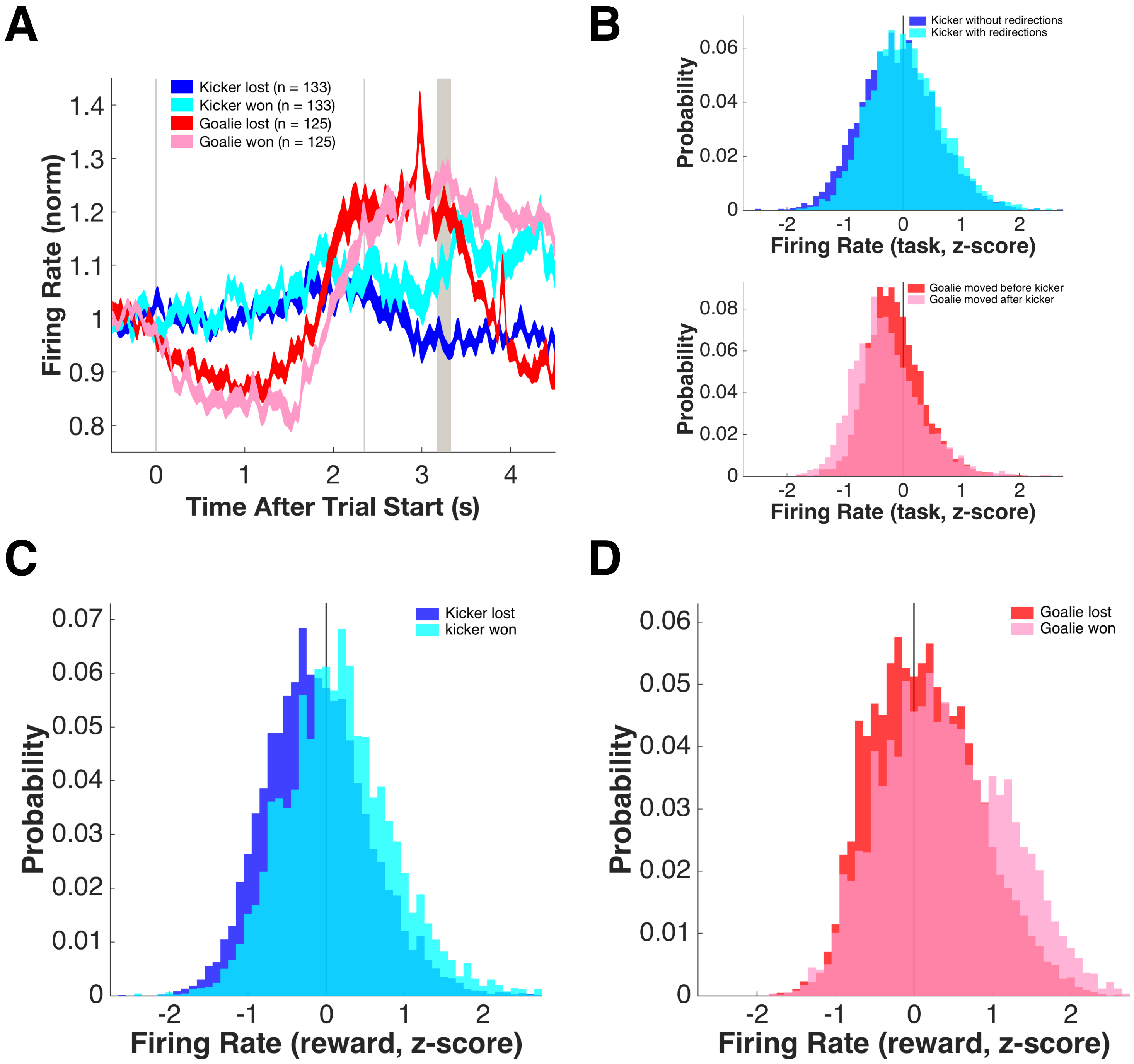


Figure4


A
Kicker lost (n = 133)
Kicker won (n = 133)
Goalie lost (n = 125)
Goalie won (n = 125)
Firing Rate (norm)
1.4
1.3
1.2
1.1
1
0.9
0.8
0
1
2
3
4
Time After Trial Start (s)
B
Kicker without redirections
Kicker with redirections
Probability
0.06
0.04
0.02
0
-2
-1
0
1
2
Firing Rate (task, z-score)
Goalie moved before kicker
Goalie moved after kicker
Probability
0.08
0.06
0.04
0.02
0
-2
-1
0
1
2
Firing Rate (task, z-score)
C
Kicker lost
kicker won
Probability
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-2
-1
0
1
2
Firing Rate (reward, z-score)
D
Goalie lost
Goalie won
Probability
0.06
0.05
0.04
0.03
0.02
0.01
0
-2
-1
0
1
2
Firing Rate (reward, z-score)

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description	
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arginine vasopressin	Aldrich	V0377		
electrode array	Probes	custom order		
Eyelink	SR Research	1000 Plus		
grid	Crist	6-YGD-J1		
joystick	CTI	M20U9T-Nx		
LCD monitor	BenQ	XL2730		
microdrive	Kopf	2650		
microsyringe	Hamilton	7633-01		
neuron sorting software	Plexon	Offline Sorter		
oxytocin	Aldrich	O4375		
pediatric nebulizer	Pari Labs	PAR310F35EACH		
primate chair	Crist	1-CC		
recording chamber	Crist	6-IAM-J05		
recording system	Plexon	OmniPlex		
single electrode	FHC	custom order		
solenoid	Crist	5-RLD-E2-C		
video camera	Logitech	C525		
X-Y stage	Crist	X-Y stage		

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Author(s): Yaoguang Jiang, Michael L Platt

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Article Title:	Using dynamic, interactive paradigms to study social neuroscience in rhesus macaque monkeys
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Editorial comments:

Changes to be made by the Author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have now thoroughly proofread the revised manuscript.

2. Please revise lines 399-402 to avoid previously published text.

This section of the Discussion has been modified accordingly.

3. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .svg, .eps, .psd, .pdf, or .ai file.

We have uploaded all the figures as individual PDF files.

4. Please provide an email address for each author.

We have provided the email address of the corresponding author in the manuscript (Yaoguang Jiang: jiangyaoguang@gmail.com). It is however unclear where in the manuscript we should put the email address of the non-corresponding author (Michael L. Platt: mplatt@pennmedicine.upenn.edu).

5. Please revise the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"

The Short Abstract has been modified accordingly.

6. Please move the ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

This has been fixed.

7. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

We have made sure the numbering system is correct.

8. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

We have made sure the protocol section conforms to journal standard.

9. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please see the following examples:

We have inserted the proper citation (Jiang & Platt 2018) for the first experiment, and added more details for the second experiment. However, we want to emphasize that some of these details are not critical for performing the protocols described. For example, the overall number of monkeys in a colony room is irrelevant as long as the monkeys participating in the experiment live in the same colony room (with or without other monkeys) for the duration of the experiment.

1.2: Please specify how many monkeys are included in this experiment.

7 adult macaque monkeys are included in this experiment.

1.3: How many monkeys are there in a colony room?

For the duration of the experiment these 7 monkeys were the only monkeys in the colony room.

2.3: How many different M2s are there? What is the duration for each M2? For how many days is this step repeated?

All 7 participating monkeys have equal probability of being M2. On each day one monkey acts as M1 and faces all the other 6 monkeys, each for 5 minutes. Each treatment condition is repeated 5 times for each M1.

3.1.2, 3.2.2, etc.: For how many days is this step repeated? Is OT or saline given to every monkey or only a specific group? Please specify throughout the protocol.

Treatment is only delivered to M1s. This has been further specified in the protocol.

3.2.2: Is the monkey restrained or anesthetized before injection?

Monkeys are restrained via a head holder fixed on the primate chair. The are not anesthetized.

Line 143: Please describe how to train the monkeys.

This is explicitly explained in the next section (2.2. initial behavior training).

Line 172: How is the eye tracking system installed?

The eye camera is mounted onto the primate chair. We suggest following the instruction provided by the supplier (Eyelink 1000 Plus, SR Research) for installation of the 'primate mount' tracking mode.

Lines 175, 198-205, 213-215: Please describe how.

175: This has been further clarified. Briefly, initial training is done through gradual behavioral shaping through reinforcement.
198-205: Proper references have been added for this procedure.
213-215: We suggest closely following the user manual provided by the supplier (Omni-Plex, Plexon Inc) for these steps.

Lines 211: Is the monkey restrained or anesthetized before performing the penetration?

Monkeys are restrained via a head holder fixed on the primate chair. The are not anesthetized.

10. Please include single-line spaces between all paragraphs, headings, steps, etc.

This has been addressed.

11. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

The relevant sections have been highlighted.

12. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.

This has been noted.

13. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

This has been noted.

14. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

The Discussion has been significantly modified accordingly. We have also incorporated both reviewers' suggestions in this section.

15. References: Please do not abbreviate journal titles.

This has been fixed.

16. Please follow the book citation example below to reformat book references:
Kioh, L.G. et al. Physical Treatment in Psychiatry. Blackwell Scientific Pubs. Boston (1988).

This has been addressed as well.

Reviewers' comments:

Reviewer #1:

This manuscript contains interesting and valuable data but it needs more work before it is acceptable for publication. The manuscript joins (for no clear reasons) two different experiments that have little connection to each other. I believe both datasets warrant a separate publication. The effect of OT and AVP on social dominance, even if relatively weak, is important to document because there is a large species-dependent discrepancy in the literature. The soccer (penalty kick) task also deserves a framework on its own as it is one of the most interesting and potentially useful social tasks that have been recently developed.

We thank the reviewer for their general enthusiasm regarding our behavioral paradigms. While we agree with the reviewer that the bigger scientific questions driving these two studies are different, we do believe the two behavioral paradigms ('face-off' and 'penalty kick') are conceptually related and thus could benefit from being presented together. Specifically, as explained in the Introduction, many existing experimental paradigms in social neuroscience are too rigid and one dimensional, thus failing to capture the interactive, dynamic, and evolving nature of real life social behavior. Both of our paradigms aim to improve upon the existing designs by featuring pairs of monkeys interacting with each other in relatively unconstrained, multi-dimensional decision spaces. The neurobiological investigations accompanying these studies (i.e. how neuropeptides modulate social dominance, and how neural activity in mSTS is correlated with strategic competition) serve more as examples of the potential *applications of these behavioral paradigms, rather than the technical emphasis of the current manuscript. We have now made this point more explicitly in Discussion.*

Both components have good content that would benefit from major revisions. In the following sections, I will treat them separately.

We will try our best to address all of the reviewer's concerns.

The OT/AVP administration experiment
In the presentation of the OT/AVP data, many details are skipped or are unclear. For example, in the results section (third paragraph) the authors should clarify the following: " ….a new colony of 7 monkeys (M1 = 3 males, M2 = 3 males, 4 females)". Does this mean that the same 3 males served both as M1 and M2 but the females were only M2? If yes, this difference has bearing on the results.

The reviewer is correct that in this dataset (OT and AVP inhalation experiment) the male monkeys acted as both M1 and M2s, whereas the females only acted as M2s. By contrast, in the first dataset (OT inhalation only), all 7 male monkeys acted as M1s as well as M2s. We have further clarified this point in the figure legend of the revised manuscript. In the published paper (Jiang & Platt 2018) we have systematically compared these two dataset and determined that including female M2s in the second dataset did not in any way significantly alter the observed behavioral effects of OT inhalation. We want to emphasize that, as far as we know, the effects of OT and AVP inhalation and injection are quite consistent as long as the monkeys receiving the treatments (M1s) are males. In contrast, in a separate manuscript (submitted) we described that the same neuropeptide treatments have very different effects when female macaque monkeys are the ones that receive these treatments.

At some point the notion of "reduction" is not supported by statistical measures. For example, the title of figure 2A- F reads: "OT and AVP inhalation and injection flattens social hierarchy" yet the difference in staring time is p = 0.098 (nearly a 10% chance), which indicates that this small difference could be obtained by chance. It would be helpful to show individual data as some monkeys may show the effect whereas others may not. This is particularly important because the effects of OT across many studies from many laboratories are small and may not manifest in all subject animals.

The reviewer's point is well understood. It is absolutely the case that the behavioral effects of neuropeptides are not consistent across individual monkeys, as reported in other animal and human research (for example see Rebuttal Figures 1-2).

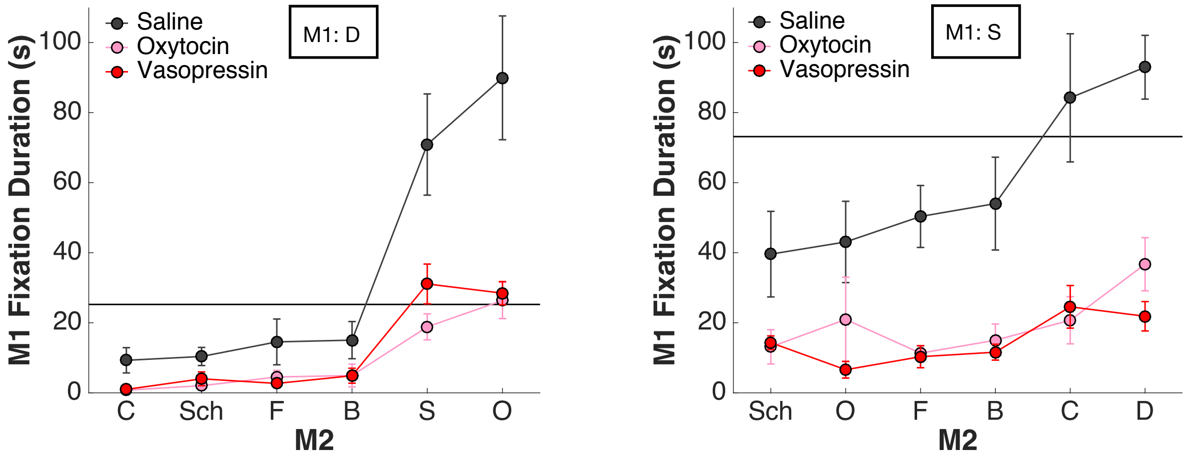

Figure 1. For the same M1 (left: D; right: S), compared with saline, OT and AVP inhalations reduce overall staring time by M1. Individuals exhibit difference in the degree of such reduction in staring. Black horizontal line: average time M1 spent staring at an empty chair; error bars: mean ± SEM.

In Jiang & Platt 2018 we have identified the main source of this individual variability to be dominance (or rather, the relative dominance between the pair of monkeys). More specifically, OT specifically reduces staring only in the most dominant M1s and M2s (for example see Rebuttal Figure 2). Even though we agree with the reviewer this nuance is very important for understanding the effects of OT and AVP administration, we do not consider this to be the most 'representative' result (per journal standard) that highlights the strength of the paradigm. We have, however, further addressed this issue in the now revised Discussion.

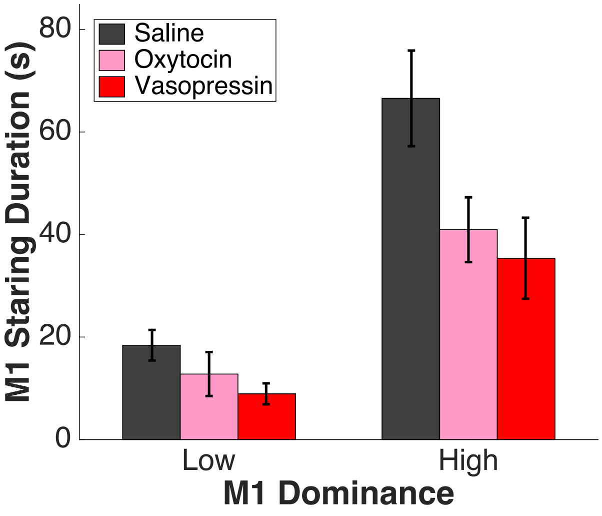

Figure 2. OT and AVP reduce staring by M1s with high dominance but not low dominance (low dominance M1, saline = 18.40 ± 2.98 s; OT = 12.78 ± 4.29 s; AVP = 8.93 ± 2.04 s; high dominance M1, saline = 66.56 ± 9.33 s; OT = 40.94 ± 6.32 s; AVP = 35.36 ± 7.91 s). Error bars: mean ± SEM.

The addition of AVP emphasizes the issue related to receptor binding/ affinity, and overall uncertainty of the mechanism that may account for the purported effects of OT on social behavior.

We completely agree with the reviewer. In fact, as mentioned in Discussion, because "…intranasal delivery of aerosolized AVP reproduces the effects of OT with greater efficacy, and that all behavioral effects are replicated when OT or AVP is injected focally into the ACCg, and "most cortical areas in primates including the ACCg lacks OT receptors but is rich in AVP receptors", we think together our results strongly indicate that "exogenous OT may shape social behavior partially through nonspecific binding with AVP receptors".

The data in figure 2 are not presented in a clear way. Although the slope estimates for the hierarchy that emerges from the analysis of pairwise staring is interesting and the plots are visually appealing, the conclusions that this is a reduction or hierarchy should be supported by statistical analysis. Overall, the ANOVA's appear to show effects but the plots are not doing justice to these data. In general, it is unclear why some of the data (that are less important) ended up illustrated in figures and other points are buried in the text.

We apologize for the confusion, but we think the reviewer may have been given an earlier version of the current manuscript. We have since significantly condensed the figures to show only the most representative results. In our opinion, both staring duration distributions (Figures 2A-B) and staring difference curves (Figures 2C-F) are equally important for understanding the main effects of neuropeptide treatment. Specifically, staring duration distributions show that neuropeptides reduced overall staring, whereas staring difference curves show that this reduction leads to a flattening of the preexisting hierarchy at both ends (i.e. the most subordinate become less subordinate whereas the most dominant become less dominant). We again refer the reviewer to our published paper for more statistical details.

The third section in the results reports a failure to replicate the effect reported in the second paragraph. OT treatment in M1 did not reduce staring in M2 yet in the previous colony (reported in the paragraph above) it did (data shown Fig 2B). This is important to address head-on and report to further emphasize that OT by itself causes little or no reliable effects. (As a matter of scientific writing, "insignificant reduction" is not a reduction at all).

We apologize for the confusion, but again we think the reviewer may be referring to an earlier manuscript, as this result is not described in the more current, condensed version of the manuscript. Nonetheless, we agree with the reviewer that there is intrinsic noise in such behavior measurements. We also think that our sample size is still relatively small, and that there may be subtle differences between the dominance hierarchy structures in different groups of subjects, which may in turn lead to different neuropeptide effects. We do want to emphasize however that in Jiang & Platt 2018, Figures 1-3 and Figures 4 were collected not only from different colonies but also in *different physical locations (the former at Duke University whereas the latter at University of Pennsylvania). We feel the fact that we could replicate most of the behavioral effects of OT inhalation in two separate colonies speaks to the validity and reliability of our behavioral paradigm.*

Figure 1A is unclear and it needs to be re-designed to better illustrate the enrollment of monkeys in OT and AVP inhalation/injection. The figure should be closer to the video #1 that shows clearly the two social partners in profile looking at or away from each other.

The reviewer's point is well understood. In the revised Figures we have chosen to keep the top-down view to illustrate the camera position (and made this point explicit in the Figure and corresponding legend), but have also inserted a profile view to better illustrate the relative positions between monkeys.

Figure 2: A and B should be labeled as inhalation and not injection trials. Why are a different number of pairs in Figure 2 C and D and E? Why aren't the slopes shown and compared?

*We apologize for the confusion, but again we think somehow the reviewer may be referring to an earlier manuscript. Regardless, the current Figures 2A-B and 2C-D do have different numbers of data points, because in Figures 2A-B each data point corresponds to one session, whereas in Figures 2C-D each data point corresponds to one unique monkey pair (n = 7*6 = 42 pairs). Figure 2E and Figure 2F also use one point to represent one monkey pair, but they have different numbers of data points from Figures 2C-D because the sample sizes are slightly different (as the first OT experiment has 7 M1s, the OT/AVP inhalation experiment has 3 M1s, whereas the OT/AVP injection experiment has 2 M1s). We refer the reviewer to our previous publication (Jiang & Platt 2018) for further statistical details.*

The ACC is a large area with multiple subdivisions that have been shown to carry our dissociable functions. Given the claim that injections of OT and AVP into the ACC caused a significant reduction in staring, the precise location of the injection would aid the interpretation of these results.

This too is explained in further detail in our previous publication (Jiang & Platt 2018). Briefly, OT or AVP were injected focally into the anterior cingulate gyrus (ACCg, not dorsal ACC or subgenual ACC), a part of ACC specifically implicated in empathy, social learning, and computations of 'other-oriented' information. We refer the reviewer to a recent review for more details on the anatomical connections and functional neurobiology of this area (Apps et al. 2016)

Finally, the treatment of this subject ignores or omits to cite several recent papers that are relevant for the ideas and the data presented here.

The reviewer's point is well understood. We have now significantly rewritten our Discussion according to the reviewers' as well as the editor's suggestions. In addition, *we refer the reviewer to our published paper for a more detailed discussion of all the relevant literature.*

The soccer game

This task is as interesting as it is difficult to train. It is remarkable that the authors managed to train two monkeys to interactively deliver and defend penalty kicks and replicate the "rules" of the game that lead to winning. The data presented in Figure 3 are convincing - the monkeys indeed discovered these rules and the cognitive process involved lend themselves perfectly for electrophysiological scrutiny. Indeed, the recordings from STS confirm that the outcome of neural recordings are only as good as the ongoing task. I think the most interesting and valuable contribution of this paper to the literature is shown in Figure 4A. The dynamic modulation of the firing rates in the course of the task in each player is worth more elaboration.

We thank the reviewer for their general positive feedback on our behavioral paradigm and its theoretical impact.

The data, however, are not presented in the best form possible. Overall the manuscript is hard to follow. For example, in Figure 3A, the cyan and blue and red and pink lines are hard to distinguish and the markers of "o" and "x" are almost invisible. This figure does not provide a good illustration of the otherwise ingenious task. Perhaps fewer trials with thicker lines and larger lettering would better serve these data.

We appreciate this critic on the graphics. This issue has been fixed per reviewer's suggestion.

Likewise, the color dots in figure 3B are not differentiable for pair 1 and 2. If the data from the two pairs are merged, the legend should not contain 2 blue and 2 pink dots; if however, the authors intended to illustrate any differences between pair 1 and 2 the colors should be clearly distinguishable.

While we understand the reviewer's point, it is somewhat a convention in awake monkey literature to distinguish task type or cell type by color while representing monkey identity using different shapes. The purpose of this practice is exactly what the reviewer has suggested, to better differentiate task/cell type, as visually grouping items by color is much easier than by shape. Most studies however will still choose to keep the monkey identity information, because some reviewers/readers will wonder how consistent certain behaviors/neural patterns are across two monkeys.

Fig 3C adequately illustrates that the kicker redirection leads more often to winning but the statistical comparison is missing. If the error bars are SEM (which should be explicitly stated in the results or figure caption) then these differences should be significantly different. The table in Fig 3D, 3E, 3F, which is an analysis of soccer strategy is irrelevant and difficult to follow.

It is stated in the corresponding figure legend that error bars in Figure 3C represents to SEM. The corresponding statistic report has been added to the main text. We have also replaced Figures 3E-F with some learning curves that we think better illustrate the dynamic and evolving nature of this game. We think Figure 3D is important and relatively straight-forward, as it clearly demonstrates that monkeys use a variety of movement combinations that are not chosen at random, but rather sensitive to the winning probability distributions, and that monkeys behave in this virtual soccer game the same way human players behave in real life soccer matches.

Figure 4. In the first plot (Fig 4A) the caption says that the gray lines indicate the beginning and end of a trial. It is unclear how these trials were aligned. Were the trials of the same duration? If yes, then the statement under 2.1.6 (general setup) is misleading and it should be stated that the trials always ended at the same time.

Yes, all trials of game play are the same duration as the X-axis speed of the ball is constant. As this is a two-player game, one of the two juicers will always go off to deliver juice reward to one of the two monkeys. As a result, the reward delivery period is always the same duration as well. We have further clarified this point in the Protocol section. Furthermore, the first two gray lines on Figure 4A mark the beginning and end of game play; reward delivery is immediately after game play (also see our response to the next question).

Fig 4 A shows very interesting data but it is unclear what happens at each time point after the second gray line that indicates the end of the trial. When does the monkey receive reward for playing the game? Are the convergent winning and losing firing rates related to reward or to having just won or lost?

The reviewer raises a good point. We have now modified this figure to better illustrate the reward period. Note that reward delivery starts as soon as one bout of play is finished, and it extends for 0.8-1.0 s (with small jittering across trials) as indicated by the third thick grey line. To answer the reviewer's question, we found most mSTS neurons do not signal trial outcome ahead of reward delivery. Most mSTS neurons start differentiate trial outcome during reward delivery and this difference in activity persists after reward delivery.

Fig 4 B The distribution of firing rates as a function of redirections for either kicker or goalie should be statistically compared. Are these significant across the population, are the different in the same neurons across redirection or lack thereof? It seems that the analyses of these interesting and unique data are unfinished. The organization of figure 4 would benefit from a different reordering (A, C, D, and B)

We have now added more statistics regarding the firing rate distributions. We have also built general linear models (GLMs) to further explore what task parameters drive mSTS firing rates. In short, as described in the manuscript, we found mSTS neurons primarily signal strategy during the task phase and trial outcome during the reward phase. In *addition, mSTS neurons are also modulated by other game-related factors such as arousal and eye gaze (see Rebuttal Figures 3).*

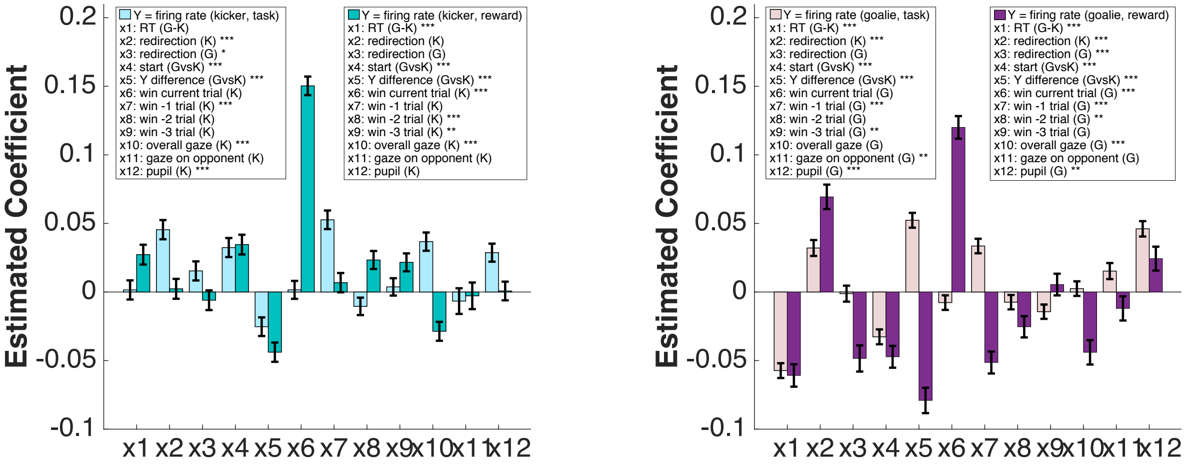

Figure 3. GLMs predicting firing rates on current trial for kicker (left) and goalie (right) neurons during live competition. Light blue, kicker task-epoch firing rate; turquoise, reward-epoch firing rate; violet, goalie task-epoch firing rate; purple, goalie reward-epoch firing rate. Error bars, estimated coefficient ± SEM.

While we have done very extensive analyses to further understand the nature of neural representation in mSTS during this game, we think the main conclusion of the current manuscript, i.e. the 'penalty kick' paradigm is a powerful, data-rich investigative tool to study dynamic social interactions, does not rely on the specific analyses performed on our electrophysiological dataset. Finally, we think Figure 4B should lead Figures C-D, as the former is about mSTS activity during task-performance, whereas the latter is about mSTS firing rate during and post reward delivery.

Overall, more detailed analyses (including more stats), more judicious decisions of what to include and not include in figures, a better anchoring of the data in the extant literature, and clearer explanation of the methods would greatly benefit both otherwise interesting and valuable studies.

We thank the review again for expressing general enthusiasm towards our paradigm. We hope the revised manuscript with new figures and additional statistic reports will address all of the remaining concerns of the reviewer.

Reviewer #2:

Manuscript Summary:
This manuscript reviews currents studies from the Platt lab that utilize interactions between monkeys to examine the social decision-making. Overall, I think this is a fair

review of their studies, but have concerns about both their broader framing of the work and some of the details of the actual studies, particularly the second experiment.

Major Concerns:
1. In terms of the 'Theoretical' framework, the authors contend that their behavioral paradigms represent natural approximations of social interactions in macaques that can be leveraged to understand the underlying neural mechanisms. This viewpoint, however, belies a misrepresentation of both the species and the paradigms being used. There is nothing 'natural' about sticking a monkey in a chair. It is a contrived setting that in turn reflects little in how monkeys would actually behave. This is not to mean that these approaches cannot inform some aspects of social cognition, but to call it natural is inaccurate. Indeed this lab has made significant contributions to our understanding economic decision making utilizing similar paradigms. Although Experiment 1 did not involve training, it is nonetheless not 'natural' to have two monkeys fixed in close proximity unable to change their location. Rhesus macaques avoid looking at each other under natural conditions, but this is the key behavioral measure for the study. Since this paper has already been published, I will not comment on the experimental details, but just make the point that it should not be characterized as reflecting 'natural' social behaviors.

We appreciate this critic from the reviewer. We have now significantly expanded our Discussion on this caveat. While it is true that all laboratory experiments, in humans or animals alike, are 'not natural' in comparison to real life, the degree to which a behavioral paradigm carries ethological validity is still a relevant question. For example, in equally controlled laboratory settings, asking a monkey to to choose between a square or a circle on the screen to indicate whether to cooperate with or betray an imaginary nonspecific is not as ethnologically meaningful as asking a monkey to choose to make eye contact with, or donate reward to, one of the other two monkeys sitting in the same room. We feel strongly that just because monkeys are seated in primate chairs (to facilitate eye movement recording, pupilometry, and electrophysiological recording), this doesn't mean one should abandon all hope and stop trying to improve upon the ethological validity of one's social paradigm. This being said, our lab also has great interest in measuring free-ranging primate behaviors in the laboratory as well as in the field. The reviewer is absolutely correct that, in comparison with naturally occurring behaviors, the best designed laboratory paradigm will still fall short in terms of flexibility and spontaneity. It is however a tradeoff one has to make from time to time, as a more restrained paradigm can afford us the opportunity to investigate more specific scientific hypotheses.

The reviewer raises a good point regarding the face-pff paradigm specifically. In our experimental setup, although the monkeys could not retreat from each other, they could look away or turn around to avoid direct confrontation. We think this relatively unconstrained but still well controlled setup affords us most of the flexibility of natural social interactions without risking actual physical contact between monkeys (for reference, see Dal Monte et al. 2016 for a very similar but more restrained setup). We have now further emphasized the caveats of this design in the revised Discussion. It is *also worth noting that, in a separate dataset (OT/AVP inhalation in females), we do have a small set of video recordings of pair-housed monkeys freely interacting with each other in the home cage in addition to our in-chair 'face-off' videos. Briefly, the free-interacting videos confirm our observation in the laboratory that neuropeptide treatment in females promotes social vigilance and aggression. While it is true that unconstrained interactions are more flexible and multi-dimensional (for example, in these videos we see monkeys approaching each other, running away from each other; or offering, accepting, or rejecting grooming), but we feel our experimental design preserves sufficient details of natural social interactions, and a slightly more unconstrained design is unlikely to alter the main conclusions of our paper.*

Finally, from a social neuroscience perspective, there is also something to be said that the closer a behavioral paradigm resembles components of real life, the more likely it is for subjects to tap into existing brain networks to solve the problem. For example, the human brain is not evolved specifically to send text messages, listen to podcasts, or play computer games remotely with other people. Yet all these activities are considered social in some way and can tap into the social brain network. Similarly, we are not suggesting monkeys play soccer with each other in the wild; instead we are arguing that our soccer task possesses enough elementary components of natural social interactions that are recognizable to monkeys that it enables us to examine the neurobiology underlying strategic social competition.

2. Since first experiment has already been published, it is reasonable that many of the key experimental details are omitted. As far as I can tell, however, Experiment 2 has not been published previously. If it has been published, the authors need to make this more obvious. Certainly this behavioral paradigm seems very intriguing. But there are simply too few details about the paradigm to accurately judge its merits. The authors present only cursory behavioral details and even less detail on the neural recordings. Without more thorough details, there is simply too little information about the study to effectively review its merits.

The reviewer's point is well understood. Review #1 has expressed similar concerns. Based on both reviewers' suggestions, we have now replaced some figures to better illustrate the dynamics nature of our task, and beefed up the Result section to include more statistics and descriptions of various aspects of the task. We did not, however, focus on expanding our neurophysiology results. While we have done very extensive analyses on this, we think the main conclusion of the current manuscript, i.e. the 'penalty kick' paradigm is a powerful, data-rich investigative tool to study dynamic social interactions, does not rely on the specific analyses performed on our electrophysiological dataset to test the hypothesis that the primate STS encodes non-perceptual social information.

Minor Concerns:
Given its relevance, I am puzzled that the Haroush & Williams study is not even cited.

We are appreciative that the reviewer has reminded us of this very reverent reference. This has been added to the revised Discussion.